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(54) Title: IMPROVING TOXICITY PROFILES IN CHEMOTHERAPY

(57) Abstract

A method of decreasing the toxicity of chemical therapeutic agents administered in cancer chemotherapy including administration to a patient undergoing chemotherapy. This reduction in toxicity can be accomplished by administering an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate. Methods of inducing mucolytic activity and reducing toxicity of acetaminophen overdose are also discussed. Such activities are induced through the administration of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

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IMPROVING TOXICITY PROFILES IN CHEMOTHERAPY

BACKGROUND OF THE INVENTION

Cancer chemotherapy has been practiced for many years with many different therapeutic agents. A major drawback of this therapy scheme is the toxicity of the chemotherapeutic agents. Agents capable of destroying invading cancer cells are unfortunately often quite toxic to normal cells. Thus, employers of and recipients of chemotherapeutic techniques have a great need for either non-toxic (to normal cells) therapeutic agents or additional agents capable of decreasing the toxicity of chemotherapeutic agents. The present invention is directed toward an agent for decreasing the toxicity of a wide spectrum of chemotherapeutic agents.

Dihydrogen phosphorothioate compounds are known to be effective as antiradiation agents. See U.S. Patent No. 3,892,824 to Piper et al and Sweeney, A Survey of Compounds from the Antiradiation Drug Development Program of the U.S. Army Medical Research and Development Command, published by the Walter Reed Army Institute of Research, Washington D.C. (1979).

Also, many disease conditions such as cystic fibrosis involve an increase in the viscosity of sputum in a patient suffering therefrom. Thus, methods of decreasing that viscosity are in demand.

SUMMARY OF THE INVENTION

The present invention involves a method for decreasing the toxicity of chemotherapeutic agents and a method for inducing mucolytic activity through the oral administration of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate. Since these chemical therapeutic agents are often administered frequently in a treatment regimen, methods for decreasing the toxicity of the same are in demand.

DETAILED DESCRIPTION OF THE INVENTION

The first aspect of the present invention is directed toward a method of decreasing the toxicity of chemical therapeutic agents administered in cancer chemotherapy comprising oral or intravenous administration to a patient undergoing said chemotherapy of an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

The second aspect of the present invention provides a method of inducing mucolytic activity to decrease the viscosity of sputum comprising oral, intravenous or inhalation administration to a patient in need of such a viscosity reduction of an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

The third aspect of the present invention provides a method of reducing hepato toxicity of acetominophen overdosage through the oral or intravenous administration to a patient in need of such a reduction of an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

By chemical therapeutic agents, there is contemplated the chemicals or compositions administered to cancer patients during the course of the patient's chemotherapy. Exemplary of such chemotherapeutic agents are alkylating agents such as cyclophosphamide, melphalan and nitrogen mustard, as well as platinum agents such as carbaplatin and cisplatin.

By mucolytic activity there is contemplated the reduction in viscosity of sputum. Such activity is important in the treatment of disease conditions that exhibit the symptom of increased viscosity of sputum. Exemplary of such conditions is cystic fibrosis.

The reduction in hepato toxicity of acetaminophen overdosage is accomplished by providing "reducing equivalents" (i.e. an external source of sulfhydryl groups). This can be accomplished through the

administration of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

By oral administration, there is contemplated the preparation of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate in any dosage form capable of oral administration. Such dosage forms include tablets, capsules, caplets, solutions and the like.

The oral dosage forms of the present invention may contain pharmaceutically acceptable inert ingredients. As such inert ingredients there are contemplated pharmaceuticals, carriers, excipients, fillers, etc. which do not interfere with the activity of the compound.

Also, fillers such as clays or siliceous earth may be utilized if desired to adjust the size of the dosage form.

Further ingredients such as excipients and carriers may be necessary to impart the desired physical properties of the dosage form. Such physical properties are, for example, release rate, texture and size of the dosage form. Examples of excipients and carriers useful in oral dosage forms are waxes such as beeswax, castor wax glycowax and carnauba wax, cellulose compounds such as methylcellulose, ethylcellulose, carboxymethylcellulose, cellulose acetate phthalate, hydroxypropylcellulose and hydroxypropylmethylcellulose, polyvinyl chloride, polyvinyl pyrrolidone, stearyl alcohol, glycerin monostearate, methacrylate compounds such as polymethacrylate, methyl methacrylate and ethylene glycol dimethacrylate, polyethylene glycol and hydrophilic gums.

Also in accordance with the present invention, there is provided a liquid-based dosage form suitable for the administration of the composition to a patient. The liquid base for this dosage form may be any liquid capable of transporting the composition into the body of a patient without disrupting the activity of the compound or harm the

patient. Exemplary of such a liquid is an isotonic solution. The isotonic solution may also contain conventional additives therein such as sugars. These solutions can be used in the preparation of oral, intravenous or inhalation composition.

Thus, the compositions of the present invention may be admixed according to known procedures using known excipients.

As an effective amount of the compound of the first aspect of the present invention, there is contemplated any amount which would serve to decrease the toxicity of chemotherapeutic agents. For example, a dosage of between about 50 to about 2500 mg/m^2 body surface area of the patient is contemplated. A preferred dosage according to the present invention is from about 300 to about 1000 mg/m^2 body surface area of the patient. The active ingredient may be administered in single or divided doses.

As an effective amount of the compound of the second aspect of the present invention, there is contemplated any amount which would serve to induce mucolytic activity in a patient in need thereof. For example, a dosage of between about 50 to about 2500 mg/m^2 body surface area of the patient is contemplated. A preferred dosage according to the present invention is from about 300 to about 1000 mg/m^2 body surface area of the patient. The active ingredient may be administered in single or divided doses.

As an effective amount of the compound of the third aspect of the present invention, there is contemplated any amount which would serve to reduce the toxicity of acetaminophen overdose. For example, a dosage of between about 50 to about 2500 mg/m^2 body surface area of the patient is contemplated. A preferred dosage according to the present invention is from about 300 to about 1000 mg/m^2

body surface area of the patient. The active ingredient may be administered in single or divided doses.

S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate can be depicted as follows:

$CH_3-NH-(CH_2)_3-NH-(CH_2)_3-S-PO_3H_2$.

S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate may be prepared in accordance with the following procedure:

Preparation of N-Methyl-N,N'-trimethylenebis-p-toluenesulfonamide (1). - A freshly prepared solution of p-toluenesulfonyl chloride (90.8 g., 0.476 mole) in N,N-dimethylformamide (200 ml.) is added during 45 min. with moderate external cooling to a stirred solution of N-methyl-1,3-propanediamine (41.9 g., 0.476 mole) in N,N-dimethylformamide (150 ml.) at such a rate that the temperature does not exceed 40°. The mixture is stirred 45 min. longer at room temperature and then poured into cold water (1.2 l.). The white gum that precipitated solidifies on standing. The crude product is collected, pulverized, and washed thoroughly with water. Recrystallization from ethanol affords the pure product, m.p. 93° (Kofler Heizbank), in 79% yield (74.4 g.).

Anal. Calcd. for $C_{18}H_{24}N_2O_4S_2$: C, 54.52; H, 6.10; S, 16.17. Found: C, 54.33; H, 5.92; S, 16.4.

Preparation of 3-Chloropropyl Acetate (2). - Acetic anhydride (114 g, 1.12 mol) is added in a thin stream to a stirred mixture of 3-chloro-1-propanol (94.5 g, 1.00 mol) and glacial HOAc (50 ml). The solution is refluxed 2 hr, cooled, and poured into H_2O (200 ml). The layers are separated, and the aqueous layer is thoroughly extracted with Et_2O (five times with 100-ml portions). The original organic layer is then combined with the Et_2O solution, and the resultant solution is washed several times with H_2O followed by saturated $NaHCO_3$ solution and finally with H_2O.

The dried ($MgSO_4$) solution is fractionally distilled under reduced pressure to give 2, bp 63-66° (12-14 mm) [G.M. Bennett and F. Heathcoat, J. Chem. Soc., 268 (1929). bp 66° (14 mm)], in 80% yield (109 g).

Preparation of Trisodium phosphorothioate. - Thiophosphoryl chloride (56.5 g., 0.333 mole) is added to a solution of sodium hydroxide (80.0 g., 2.00 moles in 500 ml. of water), and the mixture is heated with vigorous magnetic stirring to 83-84°. The heat source (Glas-Col mantle) is then immediately removed, and the mixture is quickly cooled to 75-77° by means of a water bath. When the water bath is removed, the temperature of the vigorously stirred mixture gradually rises spontaneously. The temperature is allowed to rise to 83-84°, and the mixture is again cooled rapidly back to 75-77°. This process of alternately cooling and allowing spontaneous temperature rise is repeated about six times, or until so little unreacted thiophosphoryl chloride remains that the spontaneous rise in temperature no longer occurs. The mixture, which is yellow in color, is then heated at 82-84° with continued stirring until the oily droplets of thiophosphoryl chloride disappear. [The total reaction period required is about 1 hr. As short a reaction time as possible is desired.] Immediately after the mixture becomes clear, it is chilled rapidly in an ice-water bath to about 4°. The crystalline hydrated form of the product commences precipitating when the solution becomes cold. The mixture is then allowed to stand in the refrigerator at 4° for about 16 hr. The crystalline precipitate is collected with the aid of the cold filtrate, pressed as dry as possible on the funnel, and washed with absolute ethanol (100 ml.). The precipitate is then removed from the funnel and dissolved in water (250 ml.) at 45°. The solution is filtered immediately. Absolute ethanol (200 ml.) is

gradually added with swirling to the filtrate, and the mixture is then cooled in a cold water bath to about 20°. The reprecipitated product is collected and washed with ethanol (100 ml.). The product is then dehydrated by adding it to dry methanol (600 ml.) and stirring the resultant mixture under anhydrous conditions for 1.5 hr. The white methanol-insoluble solid is collected and dried for approximately 30 min. at 100° in vacuo over phosphorus pentoxide. The anhydrous trisodium phosphorothioate thus obtained is a white powder amounting to about 50g. (83% yield), and should be stored in a freezer under anhydrous conditions.

Preparation of N-(2-Acetoxyethyl)-N'-methyltrimethylenebis-p-toluenesulfonamide (3). A solution of (1) (39.5 g., 0.100 mole) in N,N-dimethylformamide (12.5 ml.) is added during 1 hr. to a stirred suspension of sodium hydride (4.00 g. of 60% oil dispersion, 0.100 mole of NaH) in N,N-dimethylformamide (75 ml.) with moderate external cooling to maintain the temperature at about 30°. The mixture is stirred 1 hr. longer at room temperature, and a virtually clear solution results.

Freshly distilled (2) is added (13.5 g., 0.100 mole), and the resultant mixture is left to stir 42 hr. at room temperature. The mixture is then heated at 80-85° for 2 hr. Most of the solvent is removed by distillation in vacuo, and the residual red-orange sirup is dissolved in benzene (250 ml.). The benzene solution is washed with water (4 x 50 ml.) and dried (Na_2SO_4). Removal of the benzene by evaporation under reduced pressure leaves an orange oil that is used as such.

Preparation of N-(3-Bromopropyl)-N'-methyl-1,3-propanediamine Dihydrobromide (4). - A stirred mixture of crude 3 described above (46.5 g) and 48% HBr (500 ml) is refluxed overnight and then slowly distilled through a 30-

cm Vigreux column until 300 ml of distillate is collected during 8 hr. The solution that remained is cooled, treated with Norit, filter (Celite), and evaporated to dryness with aid of added portions of MeOH (aspirator, rotary evaporator, bath up to 70°). The residue is recrystallized successively from MeOH (Norit)-Et_2O and MeOH to give pure 4, mp 220-222° dec, in 40% yield (13.8 g), Anal. Calcd for $C_7H_{19}BrN_2 \cdot 2HBR$: C, 22.66; H, 5.16; Br, 64.62; N, 7.55. Found: C, 22.69; H, 5.22; Br, 64.48; N, 7.68.

Preparation of S-3-(3-Methylaminopropylamino)propyl Dihydrogen Phosphorothioate (5) Trihydrate. - Solid (4) (7.80 g, 21.0 mmol) is added in portions to a stirred partial solution of Na_3SPO_3 (3.60 g, 20.0 mmol) in H_2O (20 ml). The mixture, which soon becomes clear, is stirred at 25-30° for 1.75 hr, poured into DMF (80 ml), and refrigerated overnight. The precipitate is collected, dissolved in H_2O (20 ml), and reprecipitated by addition of EtOH. The crystalline product is collected with the aid of EtOH, washed successively with EtOH followed by Et_2O, air dried, and then equilibrated at constant 50% relative humidity to give pure (5)$\cdot 3H_2O$, mp 115-120°, in 85% yield (5.04g). Anal. Calcd for $C_7H_{19}N_2O_3PS \cdot 3H_2O$: C, 28.37; H, 8.50; N, 9.45; P, 10.45; P, 10.45; S, 10.82. Found: C, 28.35; H, 8.32; N, 9.48; P, 10.57; S, 10.91.

Preparation of 3-(3-Methylaminopropylamino)propanethiol Dihydrochloride (6). - The preparation of (5) described above is repeated (21.6 mmol of 4, 20.6 mmol of Na_3SPO_3), and the reprecipitated product (from H_2O-EtOH) is used for conversion to (6) without further characterization. The sample is dissolved in 3 N HCl (30 ml), and the solution is heated in a boiling H_2O bath for 10 min. The cooled solution is diluted with EtOH (300 ml), and Et_2O (200 ml) is added. The cloudy mixture is refrigerated overnight while crystalline solid separates. This material,

collected under N_2 and suction dried under N_2 pressure is dissolved in MeOH (100 ml), and EtOH (500 ml) is added followed by a solution of dry HCl in EtOH (3 N, 25 ml). Crystalline (6), which separates readily, is collected under N_2, washed with EtOH followed by Et_2O, and dried in vacuo (25-30°, P_2O_5); the overall yield was 58% (2.80 g), mp 244-246° dec. Anal. Calcd for $C_7H_{18}N_2S \cdot 2HCl$: C, 35.74; H, 8.57; N, 11.91; S, 13.63; SH, 14.06. Found: C, 35.59; H, 8.69; N, 11.86; S, 13.44; SH, 14.28.

Illustrative examples of the present invention follow.

EXAMPLE I

1000 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is suspended in an isotonic solution. 200 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus suspended is administered to a patient undergoing chemotherapy with cisplatin.

EXAMPLE II

500 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is suspended in an isotonic solution. 500 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus suspended is administered to a patient undergoing chemotherapy with nitrogen mustard.

EXAMPLE III

1000 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is admixed with hydroxypropylcellulose and stearyl alcohol. The mixture is then compressed into tablet form. 200 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus prepared is administered to a patient undergoing chemotherapy with cyclophosphamide, N,N-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine.

EXAMPLE IV

700 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is admixed with hydroxypropylcellulose and glycowax. The mixture is then compressed into tablet form. 500 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus prepared is administered to a patient undergoing chemotherapy with melphalan, 4-[bis(2-chloroethyl)amino]-L-phenylalanine.

EXAMPLE V

1000 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is suspended in an isotonic solution. 200 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus suspended is administered to a patient suffering from cystic fibrosis.

EXAMPLE VI

1000 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is admixed with hydroxypropylcellulose and stearyl alcohol. The mixture is then compressed into tablet form. 200 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus prepared is administered to a patient suffering from cystic fibrosis.

EXAMPLE VII

1000 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is suspended in an isotonic solution. 200 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus suspended is administered to a patient suffering from acetaminophen overdose.

EXAMPLE VIII

1000 mg of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is admixed with hydroxypropylcellulose and stearyl alcohol. The mixture is

then compressed into tablet form. 200 mg/m^2 body surface area of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate thus prepared is administered to a patient suffering from acetaminophen overdose.

Murine Toxicity Studies with S-3-(3-methylaminopropyl-amino)propyl dihydrogen phosphorothioate trihydrate

Drug Treatment (single dose)	Day 4 WBC Nadir: % Control§	Lethal Toxicity
cisplatin, 17 mg/kg i.v.	33%	80%
S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate, 750 mg/kg i.p. + cisplatin, 17 mg/kg i.v.*	84%	None
cisplatin, 12 mg/kg i.v.	52%	15%
S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate, 1000 mg/kg p.o. + cisplatin, 12 mg/kg i.v.*	82%	None

*25 minutes after S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate

§5 male $CD2F_1$ mice per group

S-3-(3-methylaminopropyl-amino)propyl dihydrogen phosphorothioate trihydrate(100 mg/ml) was dissolved at 4 degrees C in Lactated Ringer's and 5% Dextrose, pH adjusted to 7.2-7.3 with sodium bicarbonate, immediately prior to use

cisplatin was dissolved in 0.85% sodium chloride at 1.2 mg/ml

Evaluation of the Effect of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate on the Lethality of Cisplatin

Nontumored Charles River, Portage CD2F1 Female Animals

Treatment: Day 1 Only

Name	Dosage (mg/kg/dose)	Route & Schedule	Day of Death (Mean Animal Weight in Grams) 1	2	3	4	5	6	7	8	9	15	16	21	28	30-Day Surv/Tot	LD_{10} (mg/kg/dose)
Cisplatin	39	IP	(19)		1(16)		1	1	(14)	1		(20)		(21)	(22)	2/6	11
	28		(20)		(17)	1		1	3(13)			(19)		(21)	(22)	1/6	
	20		(19)		(16)				2(15)			(19)		(20)	(22)	4/6	
	14		(19)		(17)				(17)	1		(20)		(21)	(22)	5/6	
S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate																	
	1000	PO	(19)		(19)				(19)			(20)		(21)	(21)	6/6	>1000
1) Cisplatin																	
2) S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate																	
1)	39	1) IP	(19)	1	1(17)			4								0/6	18
2)	1000	2) PO, 30 min before															(Cisplatin)
1)	28	cisplatin	(19)	1	(18)				1(16)			(19)		(20)	(22)	4/6	
2)	1000																
1)	20		(20)	1	(18)				1(16)			(20)		(20)	(22)	4/6	
2)	1000																
1)	14		(20)		(18)				(16)			(20)		(20)	(22)	6/6	
2)	1000																

Cisplatin prepared in saline (soluble).

S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate trihydrate prepared in 5% dextrose lactate in Ringer's solution buffered with sodium bicarbonate to pH 7.2-7.4 (soluble).

WHAT IS CLAIMED IS:

1. A method of decreasing the toxicity of chemical therapeutic agents administered in cancer chemotherapy comprising oral or intravenous administration to a patient undergoing said chemotherapy of an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

2. A method of claim 1, wherein said S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is administered in an amount not greater than 2500 mg/m^2 body surface area of said patient.

3. A method of claim 1, wherein said S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is administered in an amount of between about 300 and 1000 mg/m^2 body surface area of said patient.

4. A method of inducing mucolytic activity to decrease the viscosity of sputum comprising oral, intravenous or inhalation administration to a patient in need of such a viscosity reduction of an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

5. A method of claim 4, wherein said S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is administered in an amount not greater than 2500 mg/m^2 body surface area of said patient.

6. A method of claim 4, wherein said S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is administered in an amount of between about 300 and 1000 mg/m^2 body surface area of said patient.

7. A method of reducing hepato toxicity of acetaminophen overdosage through the oral or intravenous administration to a patient in need of such a reduction of an effective amount of S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate.

8. A method of claim 7, wherein said S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is administered in an amount not greater than 2500 mg/m^2 body surface area of said patient.

9. A method of claim 7, wherein said S-3-(3-methylaminopropylamino)propyl dihydrogen phosphorothioate is administered in an amount of between about 300 and 1000 mg/m^2 body surface area of said patient.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US88/04445

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A61K 31/16

U.S.Cl.: 424/10; 514/629,917,922

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/10; 514/629,917,922

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

CAS-ONLINE

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	U.S., A, 3,892,824 (PIPER ET AL)01 July 1975 (01.07.85). See column 1, lines 31-37; column 2, example 2; and claim 1.	1-9
Y	Walter Reed Army Institute of Research, September, 1979 (USA), T.R. Sweeney, "A Survey Of Compounds From The Antiradiation Drug Development Program", see page 12, lines 27-28; and the Table at page 14.	1-9
Y	Cancer Clinical Trials, Volume 4, issued 1981, pages 3-6 (USA), Wasserman et al., "Differential Protection Against Cytotoxic Chemotherapeutic Effects On Bone Marrow CFU's by WR-2721", see the Introduction, page 3, and the Discussion, pages 5 and 6.	1-3 & 7-9

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

22 MARCH 1989 (22.03.89)

Date of Mailing of this International Search Report

17 APR 1989

International Searching Authority

ISA/US

Signature of Authorized Officer

Richard M. Kearse

RICHARD M. KEARSE

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	International Journal Radiation, Oncology, Biol., Phys., Volume 10, No. 9, issued September 1984, pages 1561-1564 (USA), Valeriote et al., "Dose and Interval Relationship For The Interaction Of Wr-2721 And Nitrogen Mustard With Normal And Malignant Cells", see the entire document.	1-3
Y	U.S., A, 4,424,216 (CERAMI ET AL) 3 January 1984 (03.01.84). See column 2, lines 50-65; column 3, lines 1-52; and column 6, lines 31-41.	4-6

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant; this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Chemical Abstracts, Volume 106, No. 9, issued 02 March 1987 (Columbus, Ohio, USA), Furukawa et al, "Effects Of O,O,O-tri-N-alkyl Phosphorothioates On Acetaminophene-induced Hepatotoxicity In Rats", see page 49, the Abstract No. 61084j, Toxicol. Lett. 1986, 34(1), 95-8 (Eng).	7-9
Y	U.S., A, 4,314,989 (ROSEN) 09 February 1982 (09.02.82). See column 1, lines 31-39; column 3, lines 15-36, and column 4, lines 38-67.	7-9
Y	U.S., A, 4,676,979 (SCHELLENBERG ET AL) 30 June 1987 (30.06.87). See column 1, lines 24-29.	7-9